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Physiological Diversity of Water Balance in Some African Oligochaeta

by

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SUMMARY

Oligochaeta (= few setae) are a Class that belongs to Phylum Annelida. They comprise both terrestrial (earthworms) and aquatic species. Aquatic species live in mud under the water surface. Most live in fresh water and a few are marine. This diversity of habitats involves a diversity of adaptations of water relations and water balance mechanisms. Adaptations of terrestrial Oligochaeta, when compared to their aquatic allies, are essentially adaptations to water relations at different levels of hydrature, or water content of their environments. This is reflected most clearly in the structure and function of their nephredia. The structure and functions of nephredia in higher oligochaetes differ according to whether the species is aquatic, or lives in dry, in moderately moist, or in excessively moist soil. The most simple structure and function of nephredia in higher Oligochaeta is in the aquatic African species *Alma,* where the function of the nephredium is primarily the baling out of water, which enters through the body wall by osmosis. This is comparable to the case of fish. The nephredium of the African naturalized *Aporrectodea (Allolobophora),* which lives in a wide range of soil moistures, has two functions depending on three cases of soil water content. In rather dry soil (20-25 % soil

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moisture), it conserves water by reabsorbing it from urine stored for some while in the "bladder", before ejection. This occurs apparently under hormonal control. This is comparable to the case of Amphibia. In rather wet soils (35-45 % soil moisture), the nephredium discharges excess water by increased ciliary beating and maybe also by increased water influx from the nephredial blood supply. In intermediate soil moisture conditions (25-35 %), both functions are balanced. A shift from ureotelism to ammonotelism also occurs under conditions of excessive hydration. Another outlet of nitrogenous excretions is the mucus secreted by the integument and the occasional oozing of some coelomic fluid from the dorsal pores. In the case of the African naturalized *Pheretima*, which lives in soils of 45-55% moisture content, there are two types of nephredia (septal and integumentary), with different structures and with more complicated functions, demonstrating a kind of division of functions between the two types. The function of the septal nephredium in *Pheretima* is mainly excretion of nitrogenous wastes. It draws the coelomic fluid virtually unchanged into the intestinal lumen where water and useful substances are reabsorbed while excretory substances are eliminated and are mixed with the casts. The function of the integumentary nephredium, on the other hand, is primarily the discharge of excess water and secondarily the excretion of nitrogenous wastes. Its fluid discharge may thus be better termed "perspiration". This nephredium is closed (no nephrediostome), as in the case of sweat glands, because only in this way can it prevent the escape of valuable nutritives from the coelomic fluid.

Keywords: *Allolobophora, Pheretima, Alma,* water relations, nephredial function, physiological diversity.

DEDICATION

This paper is dedicated to the memory of my teacher and mentor, Prof. Atallah Khalaf El-Duweini (1914-1979), at the Zoology Department, Faculty of Science, Cairo University, and the first oligochaetologist in Egypt and African countries.

INTRODUCTION

Oligochaeta (= few setae) are a Class that belongs to Phylum Annelida. They comprise both terrestrial (earthworms) and aquatic species. Aquatic species live in mud under the water surface. Most live in fresh water and a few are marine. This diversity of habitats involves a diversity of adaptations of water relations and water balance mechanisms.

Oligochaetes have come to be recognized as important natural resources in recent years. Since the 1950's, much interest has revolved around the utilization of earthworms as important and free agents in soil fertility (Ghabbour 1966 a), to economize on the use of chemical fertilizers, which pollute the environment. As to aquatic oligochaetes, they are widely used for feeding fish in aquaria, but what is more important is their role in development of fisheries resources in artificial reservoirs, like Lake Nasser behind the High Dam at Aswan (Ghabbour 1966 b). However, not all oligochaete species can live anywhere. There are certain definite ranges of moisture to which they are adapted (Khalaf El-Duweini and Ghabbour 1968). More recent accounts have appeared giving still more emphasis on the importance of oligochaetes in environmental management (Satchell 1983, Edwards 1998, Lavelle et al. 1999).

Adaptations of Oligochaeta to their environments are essentially adaptations to the varying moisture conditions of these habitats. Nephredia are known to perform two main functions, nitrogen excretion, and water balance. Nephredial diversity is high in Phyllodocida and ranges from protonephredia to metanephredia. An evaluation of these nephredia leads to the hypothesis that this diversity can be explained by the retainment of different stages in the transition of protonephredia into metanephredia, caused by the formation of a ciliated funnel at different ontogenetic stages (Bartolomaeus 1997). The present paper gives details about how these different species are adapted to each of these moisture ranges, through a study of the physiological adaptations of their water balance mechanisms, in which the nephredia play a major role.

Since there are many types of nephredia with a considerable variability of structure in higher oligochaetes, and many habitats that they are adapted to, an understanding of the adaptations of both aquatic and terrestrial Oligochaeta will best be achieved through an understanding of the functioning of their nephredia.

The classical example of nephredial structure and function given in textbooks, is still the one shown by the review of Edwards and Bohlen (1996), which does not differ from their second edition of 1977. It relies on the work of Ramsay (1949) and Laverack (1963), based on the nephredia of terrestrial members of the European Family Lumbricidae, particularly *Lumbricus terrestris* L. Edwards and Bohlen (1996) write that the nephredia "obviously" act as differential filters, because the composition of the urine changes as it passes along the nephredium (Ramsey 1949), but they write that it is not known whether osmotic changes are due to resorption of salt or secretion of water. Edwards and Bohlen (1996) add that urine is at a much lower osmotic pressure than the coelomic fluid, but how this is achieved is not clear. According to the same reviewers, there is some evidence that granules of waste material can be taken up by the ciliated tube of the middle wall and remain there, so that these parts of the nephredia may act as kidneys of accumulation. They add that Bahl (1947), who worked on *Pheretima*, suggested that nephredia have three functions in excretion, mainly filtration, resorption, and chemical transformation. Bahl believed that protein was reabsorbed through the nephredial wall against a concentration gradient, but according to Edwards and Bohlen (1996), this, they say, remains to be confirmed and was not accepted by Martin (1957). Again, a possible mode of functioning of the nephredia was summarized in Fig. 4.2 (after Laverack 1963), in Edwards and Bohlen (p. 77), emphasizing some of the excretion and resorption processes.

The confusion and misunderstanding shown by the review of Edwards and Bohlen (1996), were not improved from the early sixties (the date of Laverack's model), up till now, in 1999. These unfortunately still prevail for more than 30 years in textbooks about Oligochaeta. They do not take into consideration the possibility that there could be a greater diversity of nephredial structures and functions than can be known from just a few examples. They are due to the mixing of data from several species, living in several types of habitats, and belonging to several families, and from the study of several types of nephredia, as if all types of oligochaete nephredia had the same functions, and as if all oligochaetes had the same adaptations and the same water relations with their environments, and that they all should be explained by just one model.

In this paper we shall try to throw some light on this problem. Experiments described in this paper refer to comparative ecological and physiological studies performed on three species of oligochaetes having different habitat preferences and different water requirements (Khalaf El-Duweini and Ghabbour 1963, 1965, and 1968). These are:

1 - The African naturalized *Aporrectodea (Allolobophora) caliginosa* f. *trapezoides,* which can live in soils with a wide range ofwater consent ranging from 20 to 45% soil moisture,

2 - The African naturalized *Pheretima californica,* which prefers soils with a narrow, but high, soil water content, ranging from 45 to 55% soil moisture, which are levels just below water-logging, and

3- The African *Alma nilotica* and *A. stuhlmanni,* which are strictly aquatic species, living only in submerged soil (above 55% moisture),below the water's edge in the Nile and in irrigation canals and ditches.

MATERIAL AND METHODS

Species Studied:

Three oligochaete species from different habitats and with different water requirements, were chosen for the study. These were:

1 - The African naturalized *Aporrectodea (Allolobophora) caliginosa (Savigny)* f. *trapezoides* Dugès 1828, Fam. Lumbricidae, of European origin, obtained from the artificial *Eucalyptus* and *Casuarina* forest at Abu Rawwash, near Cairo (Khalaf El-Duweini 1940, Khalaf El-Duweini and Ghabbour 1963 and 1965),

2 - The African naturalized *Pheretima californica* Kinberg 1867, Megascolecidae, of south-east Asian origin, obtained from the Orman Botanical Garden in Cairo (Khalaf El-Duweini 1965 a, Khalaf El-Duweini and Ghabbour 1965), and

3 - The African *Alma nilotica* Grube 1855, endemic to Egypt and northern Sudan, and the African *Alma stuhlmanni* Michaelsen 1892, Microchaetidae, endemic to Egypt, although the genus is widespread in tropical Africa. Specimens of the larger *Alma nilotica* (25 cm long), were obtained from the Nile banks in Cairo, while specimens of the smaller *A. stuhlmanni* (15 cm long), were obtained from the irrigation canals at Abu Rawwash (Khalaf El-Duweini 1965 b, Jamieson and Ghabbour 1969, and Ghabbour 1976 a).

Points of Study:

Water relations of the three above species were studied from two inter-related points of view:

1) The effect of various environmental conditions on the organism's water relations, and

2) The physiological mechanisms involved in water balance.

A short account of the morphology of nephredia of the three species is also presented, in order to relate nephredial structure to their function in water relations. Furthermore, a number of experiments was carried out in order to elucidate the various aspects of these mechanisms. These experiments are arranged along the following lines:

A - Uptake and absorption of material:

I - Water uptake by the dry body.

II - Uptake of the phosphate ion, using P32 labeled phosphate in solution.

III - Sites of permeability.

B - Survival in solutions of various substances and in various relative humidities:

I - Survival in solutions of various concentrations of various inorganic and organic substances.

II - Changes of weight of worms in solutions of sodium chloride and glycine.

III - The effect of "brain" removal on water balance.

C - Investigations on the quantity and quality of urine (Khalaf El-Duweini and Ghabbour 1971).

D - Survival in various relative humidities (Ghabbour 1975).

E - Lipid and mineral contents of body (Ghabbour 1976 b) .

RESULTS

A - Structure of nephredia:

The dimensions of the nephredia of the three species, as given by Khalaf El-Duweini (1940, 1965 a, and 1965 b) and their relative size in comparison with the body as a whole, are not the same for the three specie. It is found that *Allolobophora* has the largest nephredial total volume, followed by *Pheretima* and *Alma.* The wide tube and the reservoir are much larger in *Allolobophora* than in *Alma,* and are absent in *Pheretima*. The septal nephredia of *Pheretima* are composed of narrow tubes only. The nephredia of *Allolobophora* and of Alma are all similar and are of the "open-exo-mega-nephric" type. *Pheretima* has a complex nephredial system made up of three types, septal, integumentary, and pharyngeal. The septal nephredia of *Pheretima* are of the "open-entero-micro-nephric" type, the integumentary nephredia are of the "closed-exo-micro-nephric" type, while the pharyngeal nephredia (which will not be dwelt upon here), are of the "closed-entero-tufted" type. This variety of nephredial types begs for an explanation.

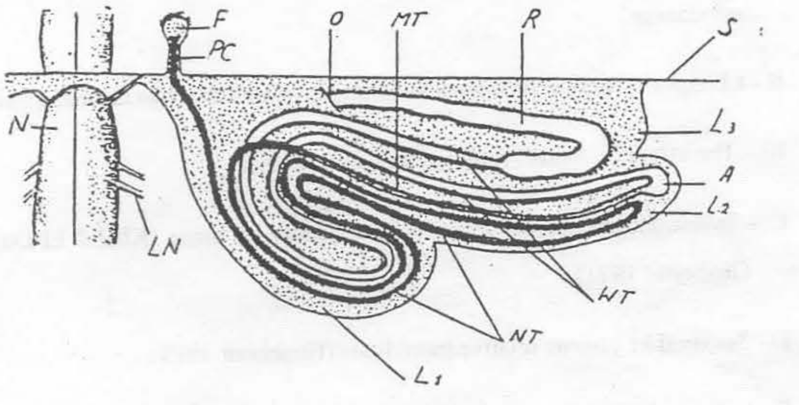


Fig 1. A diagrammatic representation of a nephridium, x25. *A:* ampulla, *F:* nephridiostome (funnel), L_1-L_2-L_3: three lobes of the nepbridial tube, *LN:* lateral nerve-trunk, *MT:* middle ciliated tube *N:* ventral nerve-cord, *NT:* narrow tube, *0:* terminal opening (nephridiopore), *PC:* preseptal canal, *R:* reservoir, *S:* septum, *WT:* wide large tube.

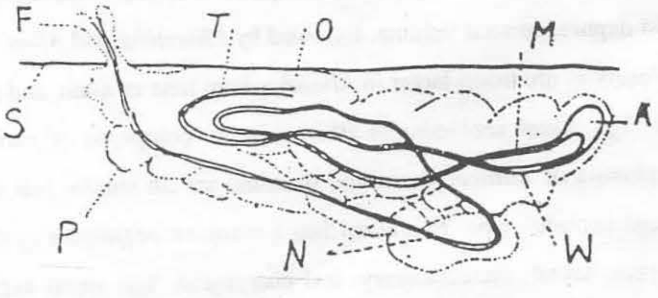


Fig 2. A semidiagrammatic representation of the nephridial tubule. A: "ampulla ". F: nephritdiostome (funnel), M: " middle" tube, N: "narrow tube" , O : nephridiopore. P: peritoneum, S: septum, T: terminal portion, W: "wide tube".

B - Uptake of material.

I - Water uptake by the dry body: *Pheretima* was found to have the highest initial rate of water uptake, but *Allolobophora* had the highest overall rate. When this was correlated with lipid content of body, it was found that *Pheretima* had the lowest lipid content. Thus, lipid content affects water uptake as a first barrier, taking into consideration that this experiment measured water uptake as a purely physico-chemical process. Rapid initial uptake is of adaptive significance for soil-dwelling worms.

II - Uptake of the phosphate ion: Worms can take up the phosphate ion labeled with P32 from its solution, indicating that they can take it also from the soil. The blood shows the highest radioactivity. *Pheretima* shows similar radioactivity in gut tissues and in the body wall. It is concluded that the phosphate ion is absorbed in earthworms from solution through the body wall.

III - Sites of permeability: Experiments done by placing worms in solutions of methylene blue, KMnO4 and AgNO3, showed that the whole surface of the body wall could be permeable. Phenol red did not appear in the gut of worms immersed in its solution, indicating that the worms do not drink the aqueous medium.

C - Survival in various media:

I - Survival in inorganic and organic solutions: Worms tolerate NaCl much less than glucose, glycine, and urea. *Alma* is always the least tolerant.

II - Weight changes in NaCl and glycine solutions: Weight changes and survival are related to the concentration of NaCl but not of glycine. Maximum loss in concentrated NaCl is 8-10% of body water but such loss is not the direct cause of death in these solutions. The cause may be the ensuing loss of salts. The mechanism of salt balance is independent of organic substances.

III - Effect of "brain" removal on water balance: "Brainless" *Allolobophora* and *Pheretima* gain much more water (15% than normal animals, whether placed in tap water or in 0.5% NaCl, after three hours of immersion. This is not the case with *Alma*. Thus "earthworms", in contrast to strictly fresh water oligochaetes, have a neuro-hormonal factor (or factors) secreted by the "brain" which seems to induce water discharge. This factor may be termed the prodiuretic factor (PDF).

IV - Survival in various relative humidities: Worms were suspended in atmospheres of constant relative humidities. The rate of water loss was proportional to the saturation deficit of the air, showing that the worms do not absorb water from a saturated atmosphere. Although *Alma* could survive for 3 days at 100% RH, yet it underwent autotomy within a few hours from the start of the experiment. *Allolobophora* had the best power of retarding water loss.

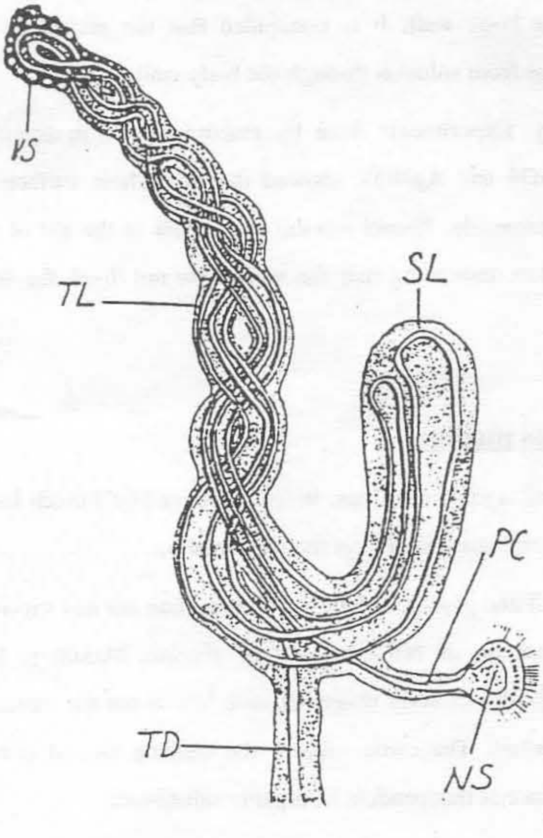


Fig 3. A semidiagrammatic representation of a septal nephridium. X210 Ns;. nephridiostome; PC. short free part of the nepbridial tube; SL. straight lobe; TD, terminal duet; TL. twisted loop; Vs. small vesicles surrcunding the tip of the twisted loop

D - Quantity and quality of urine:

· Urine was collected by 2 methods: the "funnel" and the "paraffin" methods. A reduction in the quantity of urine occurred in the "paraffin" method, due perhaps to the viscosity of the paraffin oil, which partially blocked the nephrediopores. A greater degree of reduction indicates a weaker power for the expulsion of urine. The quantities as a whole are found to be markedly less than reported by earlier workers who collected urine in saturated atmospheres. These are not the normal environments for earthworms. The lower quantities obtained in the present work are due to the fact that the "funnel" and "paraffin" methods provide unsaturated conditions. Alma produces 30% of fresh weight in 24 hrs as urine, while *Allolobophora* and *Pheretima* produce 5-7%.

In case of *Pheretima,* special attention was made to distinguish between 2 categories of excreta, the one coming with the casts via the septal nephredia and the gut; and the other (so far called "urine") coming from the integumentary nephredia.

Ammonia concentration in the casts of *Pheretima* was found to be higher than in *Allolobophora*, while urea was found to be less in the so-called urine of *Pheretima* than in the urine of *Allolobophora.* It is here suggested that the integumentary nephredia are mainly concerned with water discharge rather than with the excretion of nitrogen, and that the fluid they discharge should be better designated as "perspiration". True urine is the discharge of the septal nephredia.

E - Lipid and mineral content of body:

I - Lipid content: *Pheretima* has the lowest and *Alma* the highest lipid content. This is significant in minimizing water absorption through the body wall in the aquatic *Alma*.

II - Mineral content: Highest mineral content was found in *Pheretima* which was 3 times greater than either in *Allolobophora* or Alma. This is a further adaptation of *Pheretima* for rapid water absorption, but it leaves less disposable free solvent water to resist desiccation.

DISCUSSION

In the open nephredial system (e.g., lumbricids, *Alma,* and the septal nephredia of *Pheretima*), the coelomic fluid is drawn into the nephredial tubule by ciliary action in the funnel, the preseptal canal, the narrow tube, and the middle tube. Ramsay (1949 b) showed that in *Lumbricus,* urine is hypotonic. He also showed that almost certainly no change occurs in the composition of the coelomic fluid within the narrow tube. In the middle tube more variable results were found, but in general a slight decrease in osmotic pressure was observed. This drop was more pronounced, however, in the ampulla. At the distal end of the wide tube, the osmotic pressure was even less, suggesting that the process of forming a hypotonic urine is carried out along the entire length of the wide tube, and possibly also the middle tube. The middle tube is considered by some authors (c.f. Laverack 1963) a kidney of storage or of accumulation on account of its ability to absorb and accumulate particulate matter injected into the coelomic cavity. According to Ramsay (1949 b), the bladder seems to be solely a collecting and storage organ for the formed urine; no change in the concentration of the contents being found in relation to the wide tube.The results of the present work show, however, that *Allolobophora* under unsaturated conditions produces less urine with a higher concentration of urea than *Lumbricusin* saturated atmospheres. This change undoubtedly occurs in the bladder when it retains urine for longer periods under unsaturated conditions in order to regain part of its water into the body. Graszynski (1963) believed that the "vacuole-like hollow bodies" of the ciliated middle tube are the site of chemical transformations, e.g., absorption of glucose, and that this part of the *Lumbricus* nephredium is no kidney of storage. Again, the fine structure of the inner flat epithelium of the bladder makes it improbable that the bladder is only a reservoir and is not taking part in excretion. Its probable function may be to retain urine for some while, should there be a need for water reabsorption. This is emphasized by the fact that in *Alma* which is strictly aquatic, the bladder is almost undefined. Boroffka (1965) verified Ramsay's results and ascribed urine dilution to active absorption of ions and not to water influx (under normal conditions of hydration).

Let us now consider the functions of the nephredia of the three species of the present study in the light of the work of these authors on *Lumbricus,* and on the results of the present work. It is preferable to begin with *Alma* which is simplest both in structure and in function.

1 . The nephredium of *Alma*:

The presence of cilia in the funnel, the narrow tube, and the middle tube means active withdrawal of the coelomic fluid. The middle tube, being larger than the narrow tube, may indicate active absorption of glucose and occurrence of chemical transformation. The wide tube is hardly larger than the middle tube, thus very little, or perhaps no active uptake of salts takes place there. We may also observe that the modified investing peritoneum is in close contact with the tubule's epithelium and that there is very little connective tissue, which may indicate active expulsion of waste substances from the coelomic fluid into the tubule across the investing peritoneum. The reservoir and the sphincter are absent or undefined, a fact which shows that urine is not kept there for any length of time. In spite of this, urea concentration is similar to that of *Allolobophora,* and this is why it becomes necessary to ascribe an excretory role to the investing peritoneum.

It is evident that the main function of the *Alma* nephredium as a water balance organ is the baling out of the enormous quantities of water that are inevitably absorbed through the body wall by osmosis.

2. The septal nephredium of *Pheretima:*

Here we have the ciliated funnel and a long tube which seems in all probability to correspond as a whole to the narrow tube of *Lumbricus*. Its canal is of a uniform shape and thickness. It, however, is ciliated in certain regions, and it does not show a brownish region.The sole function of the septal nephredium, therefore, must be the withdrawal of the coelomic fluid into the intestine. The intestine would reabsorb water and useful substances such as proteins and salts. It is to be noted that the coelomic fluid of *Ph. posthuma* was not found to contain glucose, amino acids, or fats (Bahl 1947). According to Khalaf and Ghabbour (1971), larger amounts of ammonia and urea are found to be disposed of with the casts, in comparison with *Allolobophora*. The septal nephredium, therefore, helps to get rid of nitrogenous waste products and at the same time to conserve water. The function performed by the middle and wide tubes in lumbricids are thus taken over in *Pheretima* by the intestinal wall which must be also credited with the capacity of selective absorption. The urine of *Ph. posthuma* (of mixed anal and integumentary effusions), collected by Bahl (1947) from fully hydrated worms, contained 0.04 ug/100 ml amino acids. The casts of the same species also contained amino acids according to Dubash and Ganti (1964). It is quite possible that these amino acids result from the action of intestinal proteases or the action of the intestinal microflora on the proteins forwarded into the intestinal lumen by the septal nephredia. The protein present in food is digested in *Pheretima* in the crop and the anterior part of the intestine, and thus the resultant amino acids should be all absorbed before reaching the anus. The appearance of amino acids in the casts must be attributed, therefore, to the digestion of the coelomic fluid proteins passed into the intestine along its whole length via the septal nephredia.

3. The integumentary nephredium of *Pheretima* :

This closed exonephric type resembles the septal nephredia, but we cannot ascribe to it the same functions. Khalaf and Ghabbour (1968) had concluded that these nephredia, because of their structure and arrangement, are concerned with the discharge of great amounts of water. We can now elaborate on this idea and add that the integumentary nephredia go into action under conditions of excessive hydration and that their cilia would beat at an increased rate to draw in more water through two possible channels: (1) from the coelomic fluid (by a process similar to that of the flame cells of Platyhelminthes), through the walls of the nephredial tubule; (2) by influx of water from the nephredial blood capillaries. The two channels may be used simultaneously. This drawn-in fluid is eventually discharged to the outside without undergoing any changes in its composition, since no portions in the tubule corresponding to the middle and wide tubes are observed. This now explains why the integumentary nephredia are closed. A quick and copious discharge of the coelomic fluid *in toto* with its valuable content of proteins from an open nephredium would be highly injurious to the animal. It follows that we should expect to find the urea and ammonia excreted by *Pheretima* divided into two portions: one which is the main portion is present in the casts resulting from the continuous and normal action of the septal nephredia, and the other is of secondary importance and is present in the fluid discharged by the integumentary nephredia at times of excessive hydration. It is thus proposed that this latter fluid should not be described as urine, but should be given a special term. This fluid may be compared with the perspiration of mammals, since its main function is the discharge of excess water rather than the excretion of urea and ammonia, which are present however. The term "perspiration" is proposed to designate this fluid, where the integumentary nephredia are likened to sweat glands. The term "urine" is to be retained only for the fluid continuously discharged by the septal nephredia, which is concentrated in the intestine, and where it also undergoes certain chemical transformations; the components of which are eventually discharged mixed with the casts.

4. The nephredium of *Allolobophora* :

The nephredium of *Allolobophora* (as apparently in other lumbricids), is capable of two contradictory functions: (a) retention of urine and reabsorption of part of its water in case of low hydration, and this is the most normal case, compared with *Alma*, and (b) discharge of excess water in case of excessive hydration, and excessive hydration is known to be avoided by earthworms (Roots 1955 and 1956). Experiments on "brainless" worms show the presence of a neuro-hormonal control which enables the nephredium to shift from one function to the opposite according to situation. Chauchepart and Puytorac (1961) suggested that the "brain" regulates the permeability of the body wall and that its removal leads to increased permeability of the body wall. Kamemoto (1964) gave the same suggestion and added that the brain may enable the animal to eliminate water and conserve sodium. Later, Kamemoto *et al.* (1966) came to the opinion that the neurosecretory factor may be involved in nephredial function.

One of the main differences between *Alma* and *Allolobophora* is that the latter has a greater permeability of the body wall, associated with a lower lipid content. Another difference is that *Allolobophora* has a more elaborate nephredium which can cope with excessive hydration. Furthermore, a neuro-hormonal control is present in *Allolobophora* and absent in *Alma*. Thus, the normal problem with *Allolobophora* is to conserve water. This is faced by the normally functioning nephredia, which seem to have a local tendency in the bladder wall for the reabsorption of water and probably also the release of sodium. This is concluded from the fact that the function of water conservation continues to operate alone after removal of the brain. Moreover, water absorption through the body wall is advantageous for *Allolobophora,* and is increased by a lower lipid content in the normal worm, and it is not probable that the presence of the "brain" factor decreases an already increased and advantageous phenomenon. It is more reasonable to presume that the neuro-hormonal factor was developed (when lumbricids moved from aquatic to dry habitats), to counteract the local tendency in the bladder wall to conserve water, which was itself developed as a property without which an earthworm cannot live in terrestrial habitats. This is why this neuro-hormonal factor is better designated as a "prodiuretic factor". It is probable that it induces influx of water in the middle and wide tubes, in addition to inducing urine release from the bladder.

The supposed double function of the lumbricid nephredium seems to be divided between the septal and the integumentary nephredia of *Pheretima*, water conservation is taken up by the septal nephredia, and water discharge by the integumentaries. Both functions are performed simultaneously. Thus *Pheretima,* although it has a highly elaborate and dual nephredial system, remains a stenohydric form which must dwell in highly wet soils where the delicate balance between its two nephredial systems can be maintained. It cannot establish itself in soils of a low moisture content, where a form like *Allolobophora* can thrive. Both nephredial systems of *Pheretima* can function properly in highly wet soils, without disturbing the worm's water balance. In *Allolobophora*, on the other hand, the two contradictory functions of the nephredia do not operate simultaneously, and each is put to work according to the conditions of soil moisture.

As to the neuro-hormonal control of water balance in *Pheretima,* it is also probable that it enables the animal to eliminate water and is antagonistic with a local tendency to retain water, but here it must act on the integumentary nephredia and induce them to draw water from the coelomic fluid, or from the blood, to be eliminated as "perspiration".

Water balance sheets:

Table. 1 shows the water relations between *Allolobophora* and its environment in normal soil moisture conditions. This water balance sheet is derived from the results of the present study, and can be calculated from the following data:

Data Source

1. Worked soil moisture = 43.04% (Hassan et al. 1956)

2. Cast moisture = 34.90% *(Ibid.)*

3. Dry gut content as % body weight = 4.82 (Khalaf and Ghabbour 1968)

4. Period for renewal of gut contents = 20 hr (Barley 1959)

5. Net gain in water from food (ingested soil) as % of fresh weight per day

= 0.46

6. Amount of urine + mucus as % of fresh weight per day

= 6.5 (present study)

7 .Oxidative water = 75 ug/day = 0.01% of fresh weight per day

(calculated from Byzova 1965)

Therefore, the amount of water passing through each path of intake and output, and where the only unknown is osmosis, can be calculated in terms of per cent of fresh weight/day, as follows:

	Intake			Output
Ingested soil	2.48		Casts	2.02
Oxid. H2O	0.01		Urine	3.81
Osmosis	6.12	(by difference)	Mucus	2.78
Total	8.61			8.61

The percentage of water through each path is:

	Intake %			Output %
Ingested soil	28.8		Casts	23.2
Oxid. H2O	0.1		Urine	44.2
Osmosis	71.1	(by difference)	Mucus	32.2
Total	100			100

Table. 1 shows the representation of the water relations between *Pheretima* and its environment under normal soil moisture conditions, and how it differs from that of *Allolobophora*. A delicate water balance is maintained with the coelomic fluid acting as a reservoir, or a "private pond", as was suggested by Bahl (1947). The volume of this coelomic fluid can fluctuate within limits. From the Table, and a number of data, the water balance sheet of *Pheretima* can be calculated as follows:

Data	Source
1. Worked soil moisture = 37.88%	(Hassan *et al.* 1956)

2 .Cast moisture = 21.17% (*Ibid.*)

3. Dry gut contents as % body weight = 7.46 (Khalaf and Ghabbour 1968)

4. Period of renewal of gut contents = 22 hr (present study)

S. Net gain in water from food (ingested soil) as % of fresh weight per day

= 1.36

6. Amount of "perspiration" + mucus as % of fresh weight per day

= 7.58 (present study)

7. Water absorbed through body wall and oxidative water, as % of fresh

weight per day = 7.58 - 1.36 = 6.22

Therefore, the amount of water passing through each path of intake and output, and where the only unknown is osmosis, can be calculated in terms of per cent fresh weight per day as follows:

	Intake		Output
Ingested soil	3.08	Casts	1.72
Osmosis+ ox. H2 0	6.22	"Perspiration"	7.24
		Mucus	0.34
Total	9.30		9.30

These figures are all calculated as per cent fresh weight of body. When they are converted to per cent of total quantity of water handled, we obtain the following data:

	Intake %		Output%
Ingested soil	33.1	Casts	18.5
Osmosis + ox. H20	66.9	"Perspiration"	77.8
		Mucus	3.7
Total	100		100

It is evident from comparing the water balance sheets for *Allolobophora* and for *Pheretima*, that the paths of intake are almost similar, but the casts of *Pheretima* dispose of only about 18.5% of the total water handled, compared with 23% in *Allolobophora,* while the intake through food in *Pheretima* is 33.1% and in *Allolobophora* it is 28.8%. This reflects the greater absorbing power of the intestine of *Pheretima.* The mucus of *Pheretima* is much less important than in *Allolobophora.* This again reflects the greater need of *Allolobophora* for mucus in drier soils. It is then the "perspiration" of *Pheretima* which is the main pathway for outgoing water (fig. 4).

It is impossible to collect the casts of *Alma* as they are readily mixed with water, consequently, its water balance sheet could not be drawn. The water balance sheet for man is also presented here for comparison (Rapoport and Tschapek 1967):

	Intake %		Output %
Drinking	60.0	Urine	61.8
Food	27.3	Transpiration	18.2
Ox. H20	12.7	Lungs	14.6
		Faeces	5.4
Total	100		100

Comparisons with other animal groups:

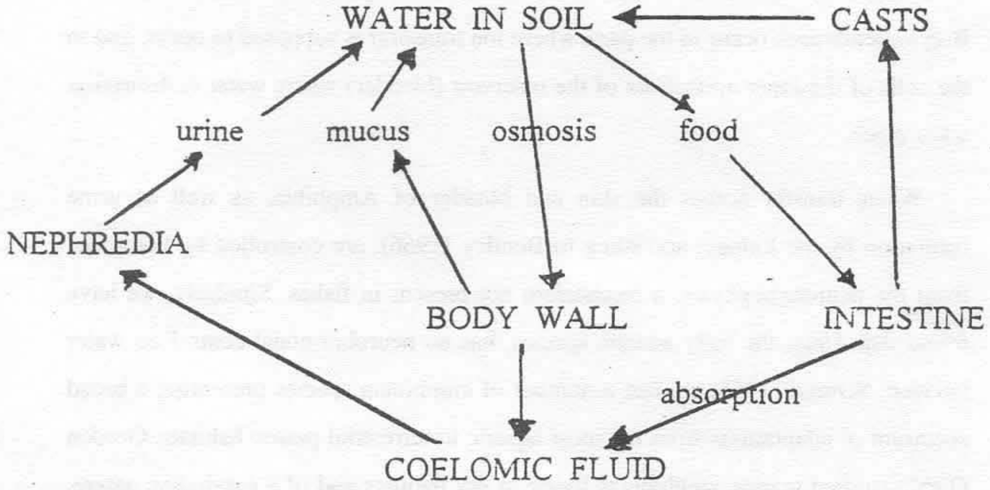
A review of the literature shows that Amphibia are the nearest group to Oligochaeta in their wafer balance mechanisms. Bentley (1966) stated that Amphibia utilize the skin to collect water from damp surfaces and to assist rapid rehydration. Extra water can be stored in and subsequently reabsorbed from the large urinary bladder, a structure which has no homologue in their ancestors, the fishes. He also added that Amphibia do not drink water and that those forms which live in arid habitats have a high tolerance for storing urea over some time and excreting it when more water is available at a subsequent time. Almost the same words can be said about the oligochaetes studied here. Again, DiBona *et al.* (1969)

found two major cell types in the mucosal epithelium of the bladder of *Bufo marinus,* the granular and the mitochondria-rich cells. This epithelium is in close contact with the basement membrane. These cells reveal fine structures similar to those found by Graszynski (1963) in the *Lumbricus* nephredium. Mitochondria and B-cytomembranes occur in the parts where ion transport is supposed to occur, and in the cells of the inner epithelium of the reservoir (bladder) where water reabsorption takes place.

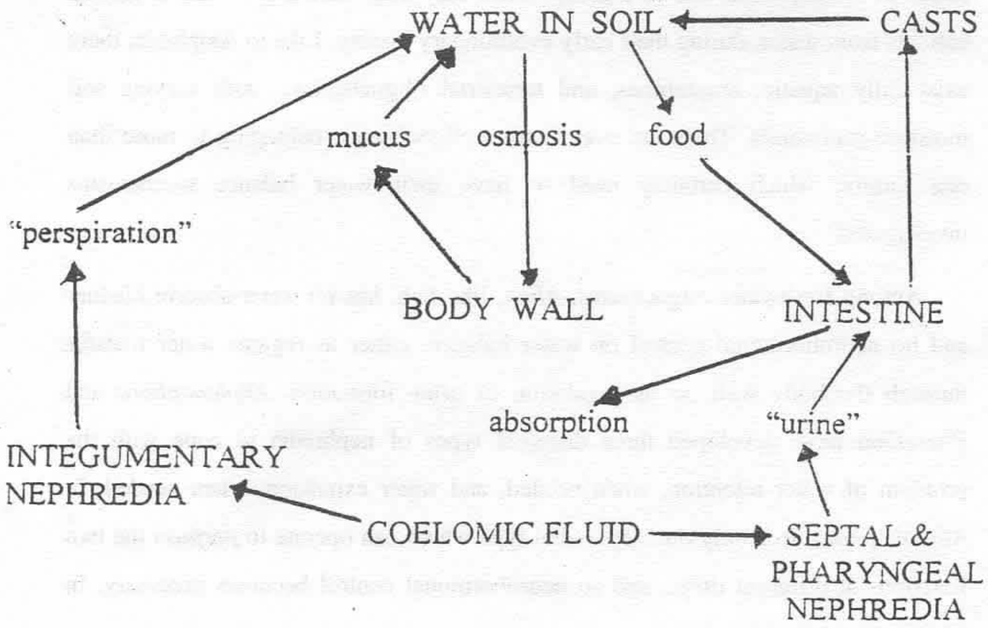
Water transfer across the skin and bladder of Amphibia, as well as urine formation by the kidney, according to Bentley (1966), are controlled by hormones from the neurohypophysis, a mechanism not present in fishes. Similarly, we have found that *Alma,* the fully aquatic species, has no neurohormonal control on water balance. Schmid (1965) studied a number of amphibian species presenting a broad spectrum of adaptations from the most aquatic to terrestrial prairie habitats. Gordon (1962) studied marine amphibians living in dry habitats and of a euryhaline nature. Cloudsley- Thompson (1967) studied the water relations of the African toad, *Bufo regularis.* These studies together indicate so many points of coincidence that we may justifiably consider the situation in Oligochaeta as a group, similar to the situation in Amphibia, also as a group, when they both started to invade terrestrial habitats from water, during their early evolutionary history. Like in Amphibia, there exist fully aquatic, amphibious, and terrestrial oligochaetes, with varying soil moisture preferanda. There are even marine oligochaetes, belonging to more than one family; which certainly need to have their water balance mechanisms investigated.

Among freshwater oligochaetes, *Alma,* like fish, has no water-absorbi bladder and no neurohormonal control on water balance, either as regards water transfer through the body wall, or as regulation of urine formation. *Allolobophora* and *Pheretima* have developed three different types of nephredia to cope with the problem of water retention, when needed, and water expulsion, when needed. In *Allolobophora,* it is only one nephredial type which can operate to *perform* the two functions at different times, and so neurohormonal control becomes necessary. In

WATER RELATIONS BETWEEN *ALLOLOBOPHORA* AND ITS ENVIRONMENT



WATER RELATIONS BETWEEN *PHERETIMA* AND ITS ENVIRONMENT



Pheretima, the two types, septal and integumentary nephredia, have divided the two functions among themselves. Instead of baling water out into the outer environment, as does the nephredium of *Alma*, the septal nephredia of *Pheretima* bail water into the intestine, where it can be more useful. Here again, a perhaps more complex neurohormonal control, that might, or should, act on the water absorptive capacity of the intestinal wall, and not only on nephredia, is also necessary.

The general conclusion that can be drawn from the present study is that earthworms, as regards their water balance mechanisms, should not be compared with fresh water animals, as was suggested by some earlier authors. Only aquatic oligochaetes like *Alma* can be compared with fresh water animals. Terrestrial oligochaetes, on the other hand, must be compared with Amphibia.

CONCLUSIONS

The following conclusions could be drawn:

1) Water balance sheets of *Allolobophora, Pheretima,* and *Alma*, are drawn up by using the data obtained from the present study and from previous literature.

2) The water relations between the species under consideration and their respective environments are totally different. Diagrams showing these relations in *Allolobophora* and in *Pheretim*a are given.

3) The function of the nephredium of *Alma,* as far as water balance is concerned, is primarily the baling out of water which enters through the body wall by osmosis.

4) The nephredium of *Allolobophora* has two functions depending on the soil water content. In rather dry soil (20-25% soil moisture) it conserves water by re-absorbing it from urine kept in the reservoir (that now could be safely considered a "bladder"), while in rather wet soil (35-45% soil moisture) it discharges excess water by increased ciliary beating and maybe water influx from the nephredial blood supply. In intermediate soil conditions (25-35% soil moisture) both functions are balanced. A shift from ureotelism to ammonotelism occurs under conditions of excessive hydration.

5) The function of the septal nephredium in *Pheretima* is mainly excretion of nitrogen. It draws the coelomic fluid virtually unchanged into the intestinal lumen where water and useful substances are re-absorbed and excretory substances are got rid of and mixed with the casts.

6) The function of the integumentary nephredia of *Pheretima* is primarily the discharge of excess water and secondarily the excretion of nitrogen. Its fluid discharge may be better termed "perspiration". This nephredium is closed because only in this way can it prevent the escape of valuable nutritives from the coelomic fluid.

7) The functions of the nenhredial blood supply are the provision of oxygen and nutritives for the active nephredia, transport of PDF, reception of water absorbed from the bladder, and influx of water in excessive hydration.

8) The septal nephredia evolved as a water conservation mechanism in *Pheretima* in dry habitats, and lost all portions beyond the narrow tube, as re-sorption of the needed water takes place in the gut. The integumentary nephredia evolved later in monsoon habitats as a water discharge mechanism on the same plan of being reduced to a narrow tube only and are closed to avoid loss of coelomic fluid in *toto*.

9) Comparison with other animal groups shows that Oligochaeta are similar to Amphibia in which is also found a development of the water absorbing bladder, increased water uptake associated with lower lipid content and a neuro-hormonal control of water balance. These developments are more apparent as one moves from the strictly fresh water species to those which live in the drier soil habitats, exposed to great fluctuations of moisture content. Therefore, only fresh water oligochaetes can be considered as fresh water animals, but the soil-dwelling oligochaetes, the earthworms, are better compared with the Amphibia. The soil-dwe~~e~~lling earthworms have developed, during the course of their evolution, more elaborate and varied nephredial systems to cope with the always changing soil moisture conditions of their environment, and as adaptations for different moisture conditions in various habitats.

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التباين الفسيولوجى فى الاتزان المائى لدى بعض قليلات الشوك الأفريقية

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ملخص

تتبع فصيلة قليلات الشوك قبيلة الحلقيات ، ومن بينها أنواع أرضية ، وأخرى مائية ، يعيش معظمها فى المياه العذبة والنادر منها فى المياه المالحة ، ولذلك توجد بينها تباينات كبيرة فى تركيب الأجهزة الإخراجية وفى آليات التخلص من المواد النتروجينية المسرفة والحفاظ على الاتزان المائى . وعند مقارنة تكيفات الأنواع الأرضية بأخواتها المائية ، نجد أن هذه التكيفات هى أساسا تكيفات للتوازن المائى عند درجات مختلفة من الرطوبة المحيطة بها فى بيئاتها . ويتبين هذا بكل وضوح فى تركيب النفريدات (أجهزة الإخراج الرئيسية) ، وفى كيفية أداء هذه النفريدات لمختلف وظائفها. ويختلف تركيب هذه النفريدات ، وتختلف كذلك وظائفها فى قليلات الشوك العليا تبعا لما إذا كان النوع يعيش فى تربة شبه جافة ، أو شبه رطبة ، أو مغمورة تماماً بالماء . ولعل أبسط هذه النفريدات تركيبا هى الموجودة فى النوع الأفريقى **ألمانيلوتيكا** ، حيث وظيفتها الأساسية التخلص من الماء الزائد الذى يدخل إلى الجسم بواسطة الضغط الأسموزى . ويمكن مقارنة هذا الوضع بذلك الموجود فى الأسماك . أما نفريدة النوع الأفريقى المتوطن **أبوريكتوديا كاليجينوزا** الذى يعيش فى تربات بها مدى واسع من الرطوبة ، فإن لها وظيفتان ويتوقف ذلك على المحتوى المائى للتربة . ففى التربة شبه الجافة (من ٢٠ إلى ٢٥٪) فهى تحتفظ بالماء بإعادة امتصاصه من البول المختزن لبعض الوقت فى « المثانة » قبل التخلص منه . والشاهد أن هذا يحدث بتحكم من الهورمونات . ويمكن أن يقارن هذا بالوضع فى البرمائيات . أما فى التربة الرطبة (٣٥-٤٥٪) فتعمل النفريدة على التخلص من

الماء الزائد بتسريع الحركة الهدبية فى فم النفريدة ، الذى يسحب من السائل السيلومى وأيضاً بتسريع سريان الماء من الدم (فى الأوعية الدموية المحيطة بالنفريدة) إلى تجويف النفريدة . أما فى التربة متوسطة الرطوبة (٢٥-٠٣٥٪) ، فإن كلتا الوظيفتين تتوازنان . كما يحدث أيضاً تحول من إنتاج اليوريا (البولينا) إلى إنتاج الأمونيا (النشادر) فى حالة الرطوبة الزائدة . وهناك مخرج آخر للمواد النتروجينية هو المخاط، الذى يفرزه الجلد ، وكذلك السائل السيلومى الذى يخرج أحياناً من الثقوب الظهرية . أما فى حالة النوع الأفريقى المتوطن **فيريتيما** ، التى تعيش فى تربات رطوبتها من ٤٥ إلى ٥٥٪ فهناك نوعان من النفريدات (الحاجزية والجلدية) لكل منهما تركيب مختلف ووظائف أكثر تعقيداً ، مما يظهر تقسيم العمل بينهما . والوظيفة الأساسية للنفريدة الحاجزية فى النوع فيريتيما التخلص من المواد النتروجينية المسرفة ، فهى تسحب السائل السيلومى بدون أى تغيير ، إلى تجويف الأمعاء التى تمتص المواد الهامة والمفيدة وتترك المواد الضارة كى تخرج مع الطرح ، أما وظيفة النفريدة الجلدية ، فهى أساسا إخراج الماء الزائد ، ويلى ذلك التخلص من المواد النتروجينية المسرفة . ولذلك يمكن أن يقارن هذا السائل الإخراجى بالعرق ، ويلاحظ أن هذه النفريدة « مغلقة » ، أى ليس لها فتحة داخلية ، مثلما هو الحال فى الغدد العرقية ، لأن هذه هى السبيل الوحيدة لمنع خروج السائل السيلومى بما فيه من مواد غذائية هامة .